spares an area midway between two portal areas, which is far removed from any rapid and straight running circulation. Mall (*5*) termed these areas *nodal points*. The oxygen tension at the nodal points is almost as low as that around the central vein. Accordingly, the enzyme pattern at these sites corresponds to that of the centrolobular area, thus promoting characteristic segmental deviations of the zonal distribution pattern (Figs. 1, right, and 2).

Since the enzymes under study reflect only part of an integrated metabolic system, it may be assumed that other chemical components reveal similar distribution patterns in the lobule. Recent reports concerning the histochemical demonstration of glucose-6-phosphatase (*6*), adenosine triphosphatase (*7*), and aminooxidase (*8*) are consistent with this assumption.

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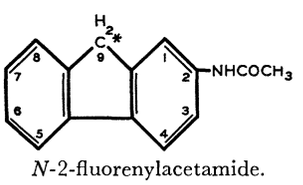
Orientation of Biochemical Hydroxylation in Aromatic Compounds

Recent reports (*1*) suggest the existence of different mechanisms for the *ortho* and *para* hydroxylation, respectively, of aromatic ring systems. Further evidence on this point can be derived from the following studies. Experiments on the metabolism of *N*-2-fluorenylacetamide in the rat, a species in which this



N-2-fluorenylacetamide.

compound is carcinogenic, have established that hydroxylation occurs at the 1, 3, 5, 7, and 8 positions (*2*). Similar methods have now been used to identify the metabolites of this compound in the guinea pig, a species in which the material failed to induce tumors (*3*).

The data of Table 1, as well as the results of other metabolic experiments (*4*), show that hydroxylation by the guinea pig takes place to a large extent at the 7 position and in an insignificant amount at positions 1, 3, 5, and 8 of the fluorene ring system. This species difference might indicate that hydroxylation at the 1, 3, and 5 positions is carried out by one enzyme system (or perhaps several systems of one type), while hydroxylation at the 7 and 8 positions could be performed by a different mechanism. Apparently the guinea pig possesses, at best, minor amounts of the former, while the rat is endowed with appreciable quantities of both.

The 1 and 3 positions are *ortho* to the substituent in the 2 position and, thus, are subject to the action of an *ortho*-hydroxylase. The 5 position is, of course, remote from the substituent in the 2 position, but it can be considered to be *ortho* to the carbon-carbon bond linking the two phenyl rings of fluorene. Thus, this position might be susceptible to hydroxylation by an enzyme system causing oxidation at the *ortho* carbon atoms. The 7 carbon atom is in an extended *para* relationship to the substituent in the 2 position (*5*). Both the guinea pig and the rat produce a relatively large amount of the 7-hydroxy derivative but only a minor amount of the 8-hydroxy derivative in the metabolism of *N*-2-fluorenylacetamide. Both compounds could conceivably originate from a common intermediate, such as the corresponding dihydrodiol, by a nonselective dehydration favoring the 7-hydroxy derivative. These data are consistent with a hypothesis that a "*para*-hydroxylase" introduces hydroxy groups into aromatic compounds via such intermediates, whereas an *ortho*-hydroxylase may do so by a different mechanism.

These results may have bearing on studies dealing with the mechanism of carcinogenesis, for *ortho*-hydroxylation of certain aromatic amines has been postulated as being involved in the carcinogenic action of these amines (*6*). The production of the *ortho*-hydroxylated derivatives of *N*-2-fluorenylacetamide in the rat (a susceptible species) and the absence of these derivatives in the guinea pig (a resistant species) lends some additional support to that hypothesis.

Table 1. Paper chromatographic identification of metabolites of *N*-2-fluorenylacetamide in rat and guinea-pig urine. Urine was collected from rats and guinea pigs that had been administered *N*-2-fluoren-9-C^{14}-ylacetamide at a dosage of 10 mg/100 g of body weight. Ether extraction of the urines to remove nonconjugated compounds was followed by incubation with β-glucuronidase to hydrolyze the glucuronic acid conjugates. The hydrolyzed mixture was extracted again with ether. This ether extract was chromatographed on S and S paper No. 598 that had been cut in the machine direction in solvent system 3 (*2*), composed of cyclohexane, *t*-butanol, acetic acid, and water (16/4/2/1 parts by volume). The spots were revealed by autoradiography on film. The 8-hydroxy derivative cannot be seen on the chromatogram of rat urine extract because of tailing from the 5-hydroxy derivative (*2*). The pattern of the free, or nonconjugated, compounds was identical with that of the glucuronic acid conjugates (*4*).

Compound	Range of R_f values (× 100) (front to back of spot)	
	Rat	Guinea pig
N-(1-Hydroxy-2-fluorenyl)acetamide	66–88	
N-(3-Hydroxy-2-fluorenyl)acetamide	44–58	
N-(5-Hydroxy-2-fluorenyl)acetamide	18–29	
N-(8-Hydroxy-2-fluorenyl)acetamide	?	17–23
N-(7-Hydroxy-2-fluorenyl)acetamide	8–14	6–17

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